Impairment of amino-acid absorption in suckling rats infected with Cryptosporidium parvum

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Abstract In the present study, we explored the nutritional consequences of cryptosporidiosis. In order to ascertain the direct responsibility of C. parvum for impairment of staturoponderal development observed during the infection in neonatal animals, we investigated the absorption of two major components of the total amino acids in dam’s milk (leucine and glutamate) across the ileal mucosa. The infection resulted in significant (47% and 34%, respectively) reductions in leucine and glutamate fluxes ($P < 0.01$). Moreover, the leucine aminopeptidase and alkaline phosphatase activities were reduced in the infected ileal mucosa. Interestingly, the reduction in weight gain, which began at day 6 post-infection (PI), persisted until day 20 PI, although no cryptosporidia were detected in the ileal mucosa after day 12 PI. We thus provide evidence that the malabsorption of amino acids during cryptosporidiosis contributes to impairing the development of neonatal animals, with consequences that persist beyond eradication of the parasite.

Introduction

Cryptosporidium parvum is now recognized as being one of the most common human enteric infections. Essentially unrecognized in humans before the development of acquired immune deficiency syndrome (AIDS), it is now established as an opportunistic agent in immunocompromised individuals and an important cause of diarrhea in infants that frequently leads to malnutrition. Infants appear to be more susceptible than adolescents and adults, and growth retardation is associated with persistent infection (O’Donoghue 1995; Clark 1999). No consistently effective treatment exists for cryptosporidiosis and an intact immune system constitutes the major factor in resolving this disease (Griffiths 1998; Clark 1999).

The pathophysiological mechanisms of cryptosporidiosis are uncertain and controversial. In the neonatal piglet, cryptosporidiosis leads to a malabsorptive diarrheal syndrome characterized by impaired intestinal glucose and glutamine-coupled Na$^+$ absorption, owing to villous atrophy, and an inflammatory infiltration of the lamina propria (Argenzio et al. 1990, 1994; Moore et al. 1995). A major impairment of neutral NaCl absorption was also observed; prostaglandin E₂ appeared to be responsible for much of this effect, acting via mechanisms that included the enteric nervous system (Argenzio et al. 1993, 1996).

In a model of symptomatic cryptosporidiosis in suckling rats, we previously demonstrated impairment of glucose transport without any involvement of prostanoids, a reduction in peroxidase absorption and a reduction in the paracellular absorption of mannitol across the ileal mucosa, suggesting the development of a malabsorptive syndrome (Capet et al. 1999).

The purpose of the present study was to investigate amino-acid absorption in suckling rats with symptomatic cryptosporidiosis, in order to ascertain the direct responsibility of C. parvum infection for the failure to thrive observed during the infection. During this study,
transport experiments were focused on the effect of acute cryptosporidiosis on the ileal absorption of leucine (Leu) and glutamate (Glu), amino acids which are abundant in the milk of the dam (10% and 20% of total amino acids, respectively) (Davis et al. 1994), and representative of bipolar and anionic amino acids, respectively. Determination of brush-border membrane hydrolases, alkaline phosphatase and leucine aminopeptidase was performed in parallel in C. parvum-infected and control rats.

Overall, our results demonstrated amino-acid malabsorption during acute cryptosporidiosis that contributed to the failure to thrive observed in newborn rats.

Materials and methods

Animals

Specific-pathogen-free (SPF) Sprague–Dawley female rats with 4-day-old litters were obtained from IFFA CREDO (L’Arbresle, Lyon, France). A single dam with its litter was housed in each separate cage.

Parasites

C. parvum oocysts were produced in a high-yield outbred suckling-rat model (Capet et al. 1999). The oocysts, initially isolated from an HIV-infected patient and purified by serial filtration followed by discontinuous sucrose gradient, as described by Arrowood and Sterling (1987), were maintained by serial inoculations in SPF Sprague–Dawley suckling rats. Briefly, purified oocysts were inoculated into intragastric gavage into 4-day-old suckling rats. The rats were killed at day 8 post-infection (PI), which is the peak time of infection, colonos were removed and the contents flushed with 1 ml of 0.150 M phosphate-buffered saline (PBS), pH 7.2. All colonic perfusates were pooled. An aliquot of 20 µl was smeared onto a glass slide and stained with the Ziehl–Neelsen reagent, according to Henriksen’s method (Henriksen and Pohlenz 1981) to quantify the infection by microscopic examination (×400). The pooled colonic perfusates were kept for 7–15 days at 4 °C in the presence of penicillin (100 IU ml⁻¹), streptomycin (100 µg ml⁻¹) and amphotericin B (0.25 µg ml⁻¹), between each inoculation.

Experimental design

A total of ten litters of control rats and ten litters of C. parvum-infected rats were used during this study. Litters were adjusted to 11 animals per dam. Experimental infection was performed in 4-day-old suckling rats by gastric intubation with 200 µl of colonic perfuse containing 10⁵ cryptosporidia. Rats from the control litters were also inoculated by gavage on day 4 with 200 µl of colonic perfuse obtained from 12-day-old SPF rats that were not inoculated with C. parvum. The parasite load of the animals used in each experiment was checked at the time of killing on a 1-cm-long segment of distal ileum, which was collected, rinsed with PBS then homogenized in 10 vol. of PBS using a glass tissue grinder. An aliquot of 20 µl was smeared onto a glass slide and stained as described above. Parasite load was determined by counting cryptosporidia in 50 high-power fields (×400). Only animals with more than five oocysts per 50 microscope fields were used in this study. Oocysts were never detected in control rats.

For flux measurement experiments, two litters of control rats and two litters of infected rats were used for each of the amino acids used in this study. Rats were weighed on days 0, 2, 4, 6, 8, and 20 PI. On day 8 PI, half of each litter was killed by chloroform asphyxiation and used for ex vivo amino-acid flux measurement; the remaining animals were bred at day 20 PI, i.e. the 8th day after spontaneous clearance of the parasite, to check whether any catch-up in growth had occurred at that date. In a second set of experiments, C. parvum-infected rats (two different litters) and control rats (two different litters) weighed on days 0, 2, 4, 6, 8, 10 PI, killed on day 8 PI, i.e. at the peak of infection, were used for the measurement of brush-border enzyme activities in three different intestinal compartments. Determination of radioactivity was performed to check for the presence of diarrhea. In a third set of experiments, C. parvum-infected rats (four different litters) and control rats (four different litters) were killed sequentially on days 4, 6, 8 and 20 PI. The whole small intestine was removed and divided into three segments of equal length and mucosal protein content was determined for the proximal and distal intestines.

Ex vivo measurements of leucine and glutamate fluxes across the ileal mucosa

Mucosal to serosal fluxes of amino acid across the intestinal mucosa were determined in ten control rats and five infected rats for leucine and in ten control rats and seven infected rats for glutamate, using ileal sheets mounted in Ussing chambers. Rats were killed on day 8 PI (peak of infection) by chloroform asphyxiation. The abdomen was opened through a ventral midline incision and the ileum removed. A 3-cm segment of ileum, adjacent to the ileo-cecal junction, was rinsed free of intestinal contents, opened along the mesenteric border, and mounted between two halves of a custom-made Ussing chamber with an aperture of 0.39 cm² (Marty Technologie, Marcilly-sur-Saone, France). The mucosal and serosal compartments of the chambers were filled with 3 ml of Ringer’s solution (pH 7.4) containing (mM): NaCl: 115, NaHCO₃: 1.2, MgCl₂: 1.2, CaCl₂: 2.4, KH₂PO₄: 0.4, K₂HPO₄: 5 and sodium pyruvate. The tissue was oxygenated by a gas lift of O₂/CO₂ (95:5) and the temperature maintained at 37 °C throughout the experiment. Transepithelial fluxes of leucine and glutamate across the ileal mucosa were measured by adding 100 µM of L-leucine or L-glutamate (Sigma Chemicals, St. Louis, USA) and 1 µCi of L-[³⁵S]leucine or L-[³⁵S]glutamate (Amersham Pharmacia Biotech, Buckinghamshire, UK) to the Ringer’s solution in the mucosal compartment of the Ussing chambers. Thereafter, 500 µl samples were withdrawn from the serosal compartment every 15 min for 60 min and replaced with an equivalent volume of fresh Ringer’s buffer. A 20 µl sample was taken from the mucosal compartment 10 min after the addition of the labelled leucine or glutamate. The amount of radioactivity was measured in the different samples by liquid scintillation counting, using a Liquid Scintillation Analyzer 2200 CA (Packard Instrument, Rungis, France); the values were used to calculate amino-acid fluxes across the ileal mucosa per cm² and per min (nmol.cm⁻².min⁻¹), for the 15- to 60-min period.

Mucosal protein and brush-border enzyme activity determination

The total protein concentration of the small intestine was measured in duplicate according to the method described by Lowry et al. (1951), on days 4, 6, 8 and 20 PI, in control and C. parvum-infected rats.

Leucine aminopeptidase (LAP) and alkaline phosphatase (ALP) activities were determined at day 8 PI in 16 and 15 of control rats, respectively, and in 16 C. parvum-infected suckling rats. The small intestine was divided into three parts (proximal, median and distal). Each segment was washed with ice-cold PBS, homogenized using a glass tissue grinder (40 s; 500 r.d/min) in 500 µl PBS, centrifuged (4°C, 10 min; 7,000 g) and the mucosal supernatant was stored at −20 °C. LAP and ALP activities were measured spectrophotometrically, in duplicate, by recording the appearance of the reaction product as a function of time (Eichholz 1967; Maroux et al. 1973). One unit was defined as the activity that hydrolyzed 1 µmol of substrate in 1 min at 37 °C. Results were expressed as milliunits (mU) per mg of protein.